SUPPLEMENTAL MATERIAL

Intellectual Impairment in School-Age Children Exposed to Manganese from Drinking Water

Bouchard MF, Sauvé S, Barbeau B, Legrand M, Brodeur M-E, Bouffard T, Limoges E, Bellinger DC, Mergler D

Measurements of manganese hair concentration

Hair samples were cleaned by sonication for 15 min in 20 mL of 1% Triton X-100 solution in a 50-mL beaker, rinsed 3 times with distilled milliQ water, and dried in a convection oven at 70 °C for 24 h. Duplicate samples of approximately 20 mg were weighed and digested with 1 mL of concentrated nitric acid and 1 mL of hydrogen peroxide (30% Suprapur®), in 7 mL teflon vials for 24 h. Samples were filtered with filter paper (Fisher Scientific, Q5), and the volume was completed with distilled-deionised water (Millipore Ultra Pure Water System, 18 mΩ•cm) to 10 mL. Reagent blanks and certified hair material (GBW 09101, Shanghai Institute of Nuclear Research) were incorporated into the preparation of each set of hair samples. Trace metal analysis (Mn, Pb, Fe, As, Zn, and Cu) was performed by inductively coupled plasma mass spectrometry (Varian ICP-820 MS). Calibration curves were run every 30 samples, together with laboratory blanks. When manganese concentrations for certified hair material were outside of the designated concentrations, the hair samples of the corresponding set were excluded from the present statistical analyses. The duplicates were highly correlated (n = 268 pairs; Pearson R = 0.97). When there was only one available measure, due to contamination or insufficient hair for duplicates, the sole measure was retained for the statistical analyses; for the others, the mean of duplicates was used.

Estimation of manganese intake from the diet and water consumption

During the home visit, a questionnaire was administered verbally to the parent and the child to assess manganese intake from the diet and water consumption. The dietary manganese intake was estimated using a 50-item monthly semi-quantitative food frequency questionnaire, which included items contributing to intake, either because they have elevated manganese content or are commonly consumed. The questionnaire was developed systematically and reviewed by questionnaire development experts at the Nutrition Research Division at Health Canada. The questionnaire was administered by trained interviewers. 3-D models of portion size were used to obtain more precise estimates for all but the first study site, thus data for these participants (n=16) were not included in the analyses on dietary intake. To calculate monthly dietary manganese intake, the portion sizes were matched to the corresponding food item, and multiplied by the manganese concentration of the item (from the online search engine of the Canadian Nutrient File); the manganese intakes from all items were summed, yielding the total in µg/month.

Manganese intake from water consumption was estimated from reported direct water ingestion, and from water incorporated in food preparations. The food frequency questionnaire included food items in which water is incorporated (e.g., juices made from concentrate, hot chocolate, etc). For each food item, the amount of water incorporated in the reported portion size was multiplied by the concentration of manganese in the water source used to prepare this food. The manganese intakes from all items were summed, yielding the total in µg/month. To estimate manganese intake from direct consumption of water, the number of glasses of water consumed was recorded in the reference time period given by the respondent, and volumes were transformed into monthly consumption. Separate questions were formulated for different water sources, i.e. bottled, tap, tap filtered with a pitcher, and tap with an attached filter. For each source of water, the amount consumed was multiplied by the measured or estimated concentration of manganese. For untreated

tap water and tap water treated with a carbon filter attached to the tap, the measured concentrations were used in the calculation. We conducted experiments to assess Mn removal efficacy for water filtered by a pitcher with activated carbon and an ion-exchange resin filter, showing an average Mn removal efficacy of 74%, which we used in our calculations. For bottled water, we attributed a concentration of 0 µg/L based on a survey of bottled water in Canada that reported a median < 1 µg/L for manganese concentration (Dabeka et al. 2002).

Reference

Dabeka RW, Conacher HB, Lawrence JF, Newsome WH, McKenzie A, Wagner HP, et al. 2002. Survey of bottled drinking waters sold in Canada for chlorate, bromide, bromate, lead, cadmium and other trace elements. Food Addit Contam 19:721-732.

Table 1s: Distributions of metals' concentrations in participant's tap water (µg/L); n=362.

Metals	Percentiles						
	Min	5	25	50	75	95	Max
Iron	0	2	15	87	182	491	1461
Arsenic	0	0.1	0.3	0.5	1.1	9.6	49.8
Copper	1	2	9	43	106	525	1156
Zinc	1	4	10	15	59	162	196
Lead	0	0	0.2	0.4	0.8	2.8	13.7